Exhibit B

Pharmacology of exenatide (synthetic exendin-4) for the treatment of type 2 diabetes

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New therapies for the long-term treatment of type 2 diabetes are needed to ameliorate declining pancreatic β-cell function. Ideally, these therapies should lower fasting and post-prandial blood glucose, produce no hypoglycemia or weight gain, cause no other limiting side effects, and reduce cardiovascular complications. Exenatide (synthetic exendin-4) is a potential therapeutic which may fulfill these criteria. Dose-ranging studies have identified an optimal dose of 0.05 to 0.2 μg/kg administered subcutaneously twice daily. Pharmacokinetic data support a pivotal study design which mitigates the transient nausea observed in early studies by including a dose initiation period of 1 month at 5 μg twice daily, followed by maintenance therapy at 10 μg twice daily. Ongoing studies suggest exenatide improves glycemic control through a combination of mechanisms discussed in this review.

Keywords AC-2993, diabetes, exenatide, glycemic control, glycosylated hemoglobin (A1C), synthetic exendin-4

Introduction

Type 2 diabetes is characterized by the emergence of post-prandial (post-meal) and subsequently, fasting hyperglycemia [1]. In most individuals, hyperglycemia results from a failure of pancreatic β-cells to secrete adequate insulin to compensate for insulin-resistance in peripheral tissues [2,3]. The increasing worldwide prevalence of type 2 diabetes mellitus has major implications for both healthcare systems and affected individuals, particularly because of the vascular complications associated with this disease.

The fraction of glycosylated hemoglobin (A1C) in the circulating red blood cells of healthy individuals without diabetes typically comprises 5 to 6% of total hemoglobin [4]. A1C levels provide an accurate indicator of average glucose concentrations in the blood for the previous 3 months. A1C values in individuals with poorly controlled diabetes generally exceed 9%. Results from the United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that a reduction in A1C levels reduced the risk of microvascular, and possibly macrovascular complications, and that any reduction in A1C levels was beneficial. In other words, there was no evidence of a glycemic threshold [5,6]. This study also confirmed that type 2 diabetes is a progressive disease characterized by a continuous loss of β-cell function, which is not slowed by current therapies. Treatment of patients newly diagnosed with type 2 diabetes usually advances in a stepwise manner beginning with lifestyle intervention (exercise and diet), followed by treatment with oral antidiabetic agents (OAA) such as metformin and a sulfonylurea, and may ultimately include insulin therapy [6-10]. Unfortunately, control of circulating glucose levels is rarely optimal, with average A1C values well above 8%, compared to normal levels of < 6% [4]. Many interventions also have dose-limiting side effects and other prescribing restrictions [9,10]. For example, non-glucose-dependent insulinotropic agents such as sulfonylureas can cause weight gain and hypoglycemia; metformin has been associated with gastrointestinal side effects and is contraindicated in individuals with impaired renal, liver and cardiac function; and thiazolidinediones have been associated with edema, weight gain and risk of heart failure in patients with suboptimal cardioventricular and hepatic functions.

These data suggest that new therapies for the long-term treatment of type 2 diabetes are required to delay, arrest or reverse declining β-cell function. Novel therapies should lower both fasting and post-prandial plasma glucose concentrations and, preferably, should not result in weight gain or hypoglycemia, cause no other limiting or unmanageable side effects, preserve or enhance β-cell function and reduce cardiovascular risk factors that lead to morbidity and mortality. Preferably, new agents should exhibit a unique mode of action, which is additive or synergistic with current therapies. Exenatide (synthetic exendin-4, AC-2993; Amylin Pharmaceuticals Inc/Eli Lilly & Co) is an investigational agent that may fulfill these criteria.

Non-clinical pharmacology

Natural exendin-4 was originally isolated from the salivary secretions of the lizard *Heloderma suspectum* (Gila monster) [11]. In the Gila monster, exendin-4 circulates following ingestion of a meal [12] and seems to have endocrine functions related to metabolic control. Exendin-4 has a 53% amino acid sequence overlap with mammalian glucagon-like peptide-1 (GLP-1). However, exendin-4 is transcribed from a distinct gene, not the Gila monster homolog of the mammalian proglucagon gene from which GLP-1 is expressed [13]. In mammals, subcutaneously injected exendin-4 is resistant to degradation by dipeptidyl peptidase-IV (DPP-IV) and has a much longer plasma half-life than GLP-1, which is degraded by DPP-IV in < 2 min [14,15]. Intravenous GLP-1 efficiently lowers plasma glucose in patients with type 2 diabetes, but must be given continuously to be effective, and is therefore impractical [16,17].

Exenatide is not an analog of GLP-1. In other words, the structure of the synthetic exendin-4 peptide was not created by sequential modification of the structure of GLP-1. The antidiabetic actions of exenatide include glucose-dependent enhancement of insulin secretion [18,19,20••,21•], glucose-dependent suppression of inappropriately high glucagon secretion [20••,22], slowing of gastric emptying [20••,23] which may be paradoxically accelerated in people with diabetes [24,25], and reduction of food intake [26,27••]. In addition, exenatide has been shown to promote β-cell

proliferation and neogenesis from precursor cells in *in vitro* and *in vivo* models [28•,29•,30•]. Data obtained in animal models also indicate that exenatide reduces food intake, suppresses weight gain and has an insulin-sensitizing effect [21•,26,27••]. At least some of these antidiabetic actions are likely to be mediated by exenatide binding to the known GLP-1 receptor [31]. These antidiabetic actions of exenatide, combined with enhanced pharmacokinetics, result in very high *in vivo* potency relative to native GLP-1 [21•,32,33] and make exenatide an attractive pharmaceutical agent.

In animal models of diabetes, a predominant acute action of exenatide is glucose-dependent insulinotropism, defined as the amplification of β-cell insulin secretion when glucose concentrations are above, but not below, the normal range [18,19]. The end result of this exenatide action is to increase the precision of glucose/insulin secretion coupling, while maintaining normal glucose-sensing control mechanisms, resulting in antihyperglycemic activity. This action of exenatide contrasts with the action of non-glucose-dependent insulin secretagogues or hypoglycemic agents, such as the sulfonylureas, which increase insulin secretion regardless of the glucose concentration [34] and thus have the potential to induce hypoglycemia [6].

While GLP-1 and exenatide appear to share certain glucose-lowering actions, not all actions of exenatide are predictable based on the known pharmacology of GLP-1. For example, GLP-1 but not exendin-4 has been shown to suppress gastric acid secretion [35]. Also, intraportal GLP-1 infusion triggers firing of the hepatic vagal afferent nerves, while exendin-4 does not [36]. Other data obtained in animal models of insulin resistance suggest that exenatide may also have an insulin-sensitizing effect [21•], although this has not yet been shown in humans [37]. Exendin-4 sensitized differentiated 3T3-L1 adipocytes to insulin-dependent glucose uptake, while GLP-1 had no effect in the same assay [38]. Exenatide may, therefore, exert at least some of its actions through a functionally different receptor than GLP-1 [36,38,39].

Clinical experience with exenatide

Phase I trials

A total of 48 healthy volunteers were enrolled in two phase I safety trials [37,40]. In the first study [40], individuals were enrolled in an escalating, single-dose, double-blind, placebo-controlled trial. Subcutaneous exenatide was generally well tolerated at doses of ≤ 0.1 μg/kg. Common adverse events were headache, nausea and vomiting, with nausea and vomiting being dose-limiting at 0.3 μg/kg. All exenatide doses increased plasma insulin. In the second study [37], individuals were enrolled in a single-blind, crossover trial involving intravenous infusion of exenatide at 0.12 pmol/kg/min, GLP-1 at 1.2 pmol/kg/min, or saline placebo. Glucose was clamped at 5.3 mM and insulin was infused to progressively increase insulin concentrations to approximately 65, then 190 and finally 700 pM. Endogenous insulin secretion was inhibited with somatostatin infusion at 120 ng/kg/min, while glucagon and growth hormone were maintained at basal levels by infusion of 0.65 and 3 ng/kg/min, respectively. No changes in circulating levels of glucose, insulin, C-peptide, glucagon or growth hormone occurred in non-diabetic humans. Cortisol levels were significantly higher during exenatide or GLP-1 infusions than during saline infusion ($p < 0.05$). Transient, mild nausea was the only side effect reported. These data support the conclusion that neither exenatide nor GLP-1 acutely enhance insulin activity in non-diabetic humans.

Phase II trials

Eight phase II trials of exenatide have been completed in 323 individuals with type 2 diabetes [20••,30•,41••,42-45]. A consistent pattern of safety and pharmacodynamics was generally observed. Dose-ranging studies identified an optimal glucose-lowering dose range of 0.05 to 0.2 μg/kg administered subcutaneously, with transient nausea and vomiting as dose-limiting adverse events. Pharmacokinetic profiles demonstrated minimal dependence on body weight, supporting continuing development of fixed dosage regimens. In addition, these trials have supported a pivotal study design strategy to mitigate the transient nausea observed in early studies by including a dose initiation period of 1 month at 5 μg twice daily, followed by a maintenance dose of 10 μg twice daily.

Modulation of plasma glucose

The two studies reported by Kolterman *et al* [20••] examined the ability of exenatide to modulate plasma glucose in patients with type 2 diabetes. Subcutaneous exenatide rapidly lowered both fasting and post-prandial plasma glucose. Moreover, the data demonstrated that exenatide was effective at lowering post-prandial glucose over the range of disease severity. The glucose-dependent insulinotropism exhibited by exenatide was best illustrated by the data obtained in the fasting state. There was a dose-dependent rise in serum insulin concentrations within the first 3 h after exenatide administration compared with placebo ($p < 0.001$). In sharp contrast, placebo treatment resulted in relatively stable insulin concentrations throughout the 8 h period of observation. The rise and peak of serum insulin concentrations following exenatide administration coincided with the rapid decline of fasting glucose concentrations. After 3 to 4 h post-dose, and coincident with reaching glucose nadir, mean serum insulin returned to baseline with little difference among groups. Insulin $AUC_{0\text{-}3\,h}$ and C_{max} values for all exenatide treatments increased in an apparently dose-dependent manner compared with placebo. Since exenatide concentrations remained elevated throughout the course of the assessment, consistent with the long circulating half-life of exenatide [33], the reduction in insulin beyond 3 h does not reflect a simple waning of exenatide effect due to lower circulating concentrations of exenatide. Because of the multiple acute glucose lowering actions of exenatide in the post-prandial period, it was not possible to ascertain the net individual contributions of glucose-dependent insulinotropism, suppression of glucagon secretion and slowing of gastric emptying on post-prandial glucose control.

Circulating glucagon was reduced by exenatide in both the fasting and post-prandial states. This observation supports the theory that suppression of glucagon secretion is not merely related to the slowing of nutrient presentation to the small intestine (gastric emptying). Given the well-documented elevations in fasting and post-prandial glucagon levels in patients with type 2 diabetes [46] and the

known activity of glucagon with respect to maintaining hepatic glucose output [47], it is reasonable to extrapolate that glucagon suppression by exenatide contributed to the overall effect of lowered plasma glucose in both the fasting and post-prandial periods. Like insulin, glucagon concentrations also returned toward baseline beyond 3 h post-injection during the fasting state. As the changes in insulin and glucagon concentrations are coincident and reciprocal, the effects of exenatide on the enhancement of insulin secretion and the suppression of glucagon secretion may be considered glucose-dependent. It is noteworthy that non-glucose-dependent secretagogues, as well as exogenous insulin, do not suppress the paradoxical post-prandial glucagon rise observed in patients with diabetes [48]. This results in an inappropriately low insulin-to-glucagon ratio in the portal vein (to a greater extent than with exogenous insulin), contributing to sustained rates of excess hepatic glucose production [49]. Thus, by virtue of its ability to enhance endogenous insulin and lower glucagon secretion, exenatide would tend to re-establish a more physiological and favorable portal vein ratio of insulin-to-glucagon compared with currently available agents.

Gastric emptying was also slowed by exenatide. Delivery of nutrients from the stomach to the small intestine is a critical contributor to post-prandial glucose excursions [24,50]. Indeed, non-diabetic patients who have undergone gastrectomy exhibit post-prandial hyperglycemia in spite of apparently normal β-cell function and fasting euglycemia [51]. Whether gastric emptying rates are slow, normal or accelerated in patients with diabetes without severe autonomic neuropathy is controversial [24]. One of the main confounders in understanding the pathophysiology of gastric emptying in diabetes is hyperglycemia itself, as elevated glucose concentrations in non-diabetics slow the gastric emptying rate [24]. Thus a 'normal' gastric emptying rate in the face of hyperglycemia may be considered pathophysiological, as it is relatively accelerated compared with an expected normal slowing in the face of hyperglycemia. Meal entrained, endogenous entero-pancreatic hormones such as cholecystokinin, amylin and GLP-1 slow gastric emptying, attesting to the importance of this function for overall nutrient assimilation [52]. Consistent with this observation, exendin-4 has been reported to acutely reduce food intake in healthy humans [53] and cause weight loss in animal models of obesity [21•].

Hemoglobin A1C goals

Fineman *et al* [41••] reported impressive findings in a phase II study of exenatide in patients with type 2 diabetes not attaining A1C goals (≤ 7%) with oral sulfonylureas and/or metformin and/or diet modification. Exenatide treatment for a period of 28 days reduced A1C levels by approximately 0.9% compared with baseline ($p \leq 0.006$). In addition, the proportion of patients achieving A1C ≤ 7% [54] was 4-fold greater after exenatide treatment. Given that A1C only fully reflects a change in glycemia 3 months after a sustained change has occurred, this reduction in A1C levels and enhanced ability to achieve clinically relevant A1C target values after only 1 month of therapy is highly clinically significant. Glucose profiles during ingestion of a mixed meal demonstrated that the marked, acute ability of exenatide to reduce post-prandial glycemia was sustained over the 28-day observation period. This post-prandial effect is likely to be mediated via three key actions of exenatide: (i) increased release of insulin and amylin from the β-cell [19]; (ii) suppression of the paradoxically high glucagon secretion in patients with diabetes [22]; and (iii) slowing of the rate of gastric emptying [23]. The ability of exenatide to reduce fasting plasma glucose has previously been reported to be secondary to enhanced insulin secretion [19] and suppression of inappropriately elevated glucagon secretion [22]. The effects of exenatide on both fasting and post-prandial glycemia were the key factors leading to the observed marked changes in A1C levels and fructosamine (another indicator of cumulative glycemic control). Exenatide therapy resulted in fructosamine concentrations approaching the upper limit of normal, and A1C reductions of approximately 0.9%. Such marked improvement is difficult to achieve with the simple addition of a second or third oral antidiabetic agent [55]. Moreover, while insulin therapy can be used to achieve this outcome, a vast literature documents that this approach is generally associated with significant weight gain [6], increased hypoglycemia [6], and the attendant morbidities [56].

Homeostasis model assessment

Homeostasis model assessment (HOMA) [54] was conducted to assess β-cell function at baseline and at days 14 and 28. HOMA analysis revealed improved β-cell secretory function following exenatide therapy. It is noteworthy that fasting values of plasma glucose and insulin, which were used to calculate HOMA, were obtained prior to the morning dose of exenatide when plasma concentrations of exenatide were negligible, suggesting a fundamental alteration in β-cell function following sustained exenatide exposure. These data are in keeping with the extensive literature documenting enhanced β-cell function following treatment with exendin-4 in various animal models of diabetes [28•,29•,30•].

Side effects

The most common adverse events encountered with exenatide therapy were mild-to-moderate nausea and hypoglycemia. Nausea tended to occur mostly upon initiation of therapy and subsided over the first week. Importantly, hypoglycemia was only reported in patients treated with sulfonylurea agents. While exenatide on the first day of treatment was associated with a small, acute and transient rise in serum cortisol concentrations, similar to that seen with GLP-1 treatment [55], assessment on day 28 revealed no such rise in any of the study patients. There were no clinically relevant effects of exenatide treatment on other clinical laboratory analytes, blood pressure or heart rate.

These data demonstrated for the first time [41••] that in a randomized, double-blinded trial, exenatide administered twice or three times daily for 28 days to patients with type 2 diabetes failing sulfonylurea and/or metformin, causes a marked reduction in A1C levels. A recent report [56] examined the impact of 1 month of subcutaneous exenatide dosing on blood glucose levels in poorly controlled, community dwelling, insulin-naive patients with type 2 diabetes. Even in this limited study with no placebo control, exenatide lowered A1C levels.

Conclusion

Exenatide is a promising, unique therapeutic with a novel mechanism of action and the potential to significantly improve glycemic control in patients with type 2 diabetes. Evidence suggests that exenatide achieves this improvement in glycemic control through a combination of mechanisms, which include glucose-dependent stimulation of insulin secretion, suppression of glucagon secretion, slowing of gastric emptying, inhibition of food intake and enhancement of β-cell function.

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